ANALYTICAL REPORT

Nitracaine (C16H24N2O4)
3-(diethylamino)-2,2-dimethylpropyl 4-nitrobenzoate

Remark – other NPS detected: none

Sample ID: 1443-16
Sample description: powder - white
Sample type: collected /Institute of Forensic medicine, University Freiburg, Germany
Date of sample receipt (M/D/Y): 1/14/2016
Date of entry (M/D/Y) into NFL database: 8/25/2016
Report updates (if any) will be published here: http://www.policija.si/apps/nfl_response_web/seznam.php

Substance identified - structure (base form)

Systematic name 3-(diethylamino)-2,2-dimethylpropyl 4-nitrobenzoate

Other names N,N-diethyl-2,2-dimethyl-3-((4-nitrobenzoyloxy)propan-1-amin
Formula (per base form) C16H24N2O4
M_w (g/mol) 308,38
Salt form/anions detected chloride
StdInChIKey SPTIETJWCCJSE-UHFFFAOYSA-N
Compound Class Others
Other NPS detected none
Add.info (purity..) pure by HPLC-TOF, GC-MS, NMR

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1 This report has been produced with the financial support of the Prevention of and Fight against Crime Programme of the European Union (grant agreement number JUST/2013/ISEC/DRUGS/AG/6413). The contents of this report are the sole responsibility of the National Forensic Laboratory and can in no way be taken to reflect the views of the European Commission.

2 Created by OPSIN free tool: http://opsin.ch.cam.ac.uk/ DOI: 10.1021/ci100384d

Stran 1 od 5

ID 1443-16
Report updates

<table>
<thead>
<tr>
<th>date</th>
<th>comments (explanation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
</tbody>
</table>

**Instrumental methods** (if applied) in NFL

1. **GC-MS** (Agilent): GC-method is RT locked to tetracosane (9.258 min). Injection volume 1 ml and split mode (1:50). Injector temperature: 280°C. Chromatographic separation: on column HP1-MS (100% dimethylpolysiloxane), length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm. Carrier gas He: flow-rate 1.2 ml/min. GC oven program: 170°C for 1 min, followed by heating up to 190°C at rate 8°C/min, then heating up to 293°C at a rate of 18°C/min, hold for 6.1 min, then heating at 50°C/min up to 325°C and finally 6.1 min isothermal. MSD source EI = 70 eV. GC-MS transfer line T= 235°C, source and quadropole temperatures 280°C and 180°C, respectively. Scan range m/z scan range: from 50 (30 until 6 min.) to 550 (300 until 6 min) amu.

2. **HPLC-TOF** (Agilent): 6230B TOF with Agilent 1260 Infinity HPLC with binary pump, column: Zorbax Eclipse XDB-C18, 50 x 4.6 mm, 1.8 micron. Mobile phases (A) 0.1% formic acid and 1mM ammonium formate in water; (B) 0.1% formic acid in methanol (B). Gradient: starting at 5% B, changing to 40% B over 4 min, then to 70% over 2 min and in 5 min to 100%, hold 1 min and back to 5%, equilibration for 1.7 min. The flow rate: 1.0 ml/min; Injection volume 1 µl. MS parameters: 2GHz, Extended Dynamic range mode to a maximum of 1700 amu, acquisition rate 1.30 spectra/sec. Sample ionisation: by Agilent Jet Stream technology (Dual AJS ESI). Ion source: positive ion scan mode with mass scanning from 82 to 1000 amu. Other TOF parameters: drying gas (N2) and sheath temperature 325°C; drying gas flow rate 6 l/min; sheath gas flow rate 8 l/min; nebulizer 25 psig; Vcap. 4000 V; nozzle 2000 V; skimmer 65 V; fragmentor 175 V and Octopole RF 750 V.

3. **FTIR-ATR** (Perkin Elmer): scan range 4000-400 cm⁻¹; resolution 4 cm⁻¹

4. **GC-(MS)-IR** condensed phase (GC-MS (Agilent) & IR (Spectra analyses-Danny))
   MSD source EI = 70 eV. GC-MS transfer line T= 235°C, source and quadropole temperatures 280°C and 180°C, respectively. Scan range m/z scan range: from 50 (30 until 6 min.) to 550 (300) amu.
   IR (condensed (solid) phase): IR scan range 4000 to 650, resolution 4 cm⁻¹.

5. **IC** (anions) (Thermo Scientific, Dionex ICS 2100), Column: IonPac AS19, 2 x 250mm; Eluent: 10mM from 0 to 10 min, 10-58 mM from 10 to 40min; Flow rate: 0.25 ml/min; Temperature: 30°C; Suppressor: AERS 500 2mm, suppressor current 13mA; Inj. Volume: 25 µl
## Supporting information

### Solubility

<table>
<thead>
<tr>
<th>Solubility in</th>
<th>Result/Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>soluble</td>
</tr>
<tr>
<td>MeOH</td>
<td>soluble</td>
</tr>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>soluble</td>
</tr>
</tbody>
</table>

### Analytical techniques

<table>
<thead>
<tr>
<th>Analytical technique:</th>
<th>Applied</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-MS (EI ionization)</td>
<td>+</td>
<td>NFL GC-RT (min): 8,05 BP(1): 86; BP(2): 87, BP(3): 58, (sample extracted in CH2Cl2)</td>
</tr>
<tr>
<td>HPLC-TOF</td>
<td>+</td>
<td>Exact mass (theoretical): 308,1736; measured value Δppm: -0,52; formula: C16H24N2O4</td>
</tr>
<tr>
<td>FTIR-ATR</td>
<td>+</td>
<td>Direct measurement (sample as received)</td>
</tr>
<tr>
<td>FTIR (condensed phase) always as base form</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>IC (anions)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>NMR (in FKKT)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>validation</td>
<td></td>
<td>GC-MS spectrum in good agreement by those published in EMCDDA EDND database.</td>
</tr>
<tr>
<td>other</td>
<td></td>
<td>Tranesterification in GC-MS was reported by the Hungarian Institute of Forensic Science if the sample is extracted in MeOH (see EMCDDA EDND database).</td>
</tr>
</tbody>
</table>
ANALYTICAL RESULTS

MS (EI)

Abundance

Scan 1615 (8.048 min): Nitracain_HCl_1443-16.D\data.ms

m/z ->
FTIR-ATR - direct measurement (sample as received)

IR (condensed phase – after chromatographic separation)
TOF REPORT

Data File: Nitracaine_1443-16_TOF.d
Sample Type: Sample
Instrument Name: 6230B TOF LC-MS
Acq Method: general-152105-XDB-C18-ESI-poz-pod.m
IRM Calibration Status: Success
Comment: extract in MeOH

Compound Table

<table>
<thead>
<tr>
<th>Label</th>
<th>Compound Name</th>
<th>MFG Formula</th>
<th>Obs. RT</th>
<th>Obs. Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cpd 1: Nitracaine</td>
<td>Nitracaine</td>
<td>C16 H24 N2 O4</td>
<td>5.892</td>
<td>308.1738</td>
</tr>
</tbody>
</table>

Name | Obs. m/z | Obs. RT | Obs. Mass | DB RT | DB Formula | DB Mass | DB Mass Error (ppm) |
--- | -------- | -------- | ----------- |-------|-----------|--------|-------------------|
Nitracaine | 309.1809 | 5.892 | 308.1738 | 5.9 | C16 H24 N2 O4 | 308.1736 | -0.52 |

Compounds Chromatograms

MFE MS Zoomed Spectrum

<table>
<thead>
<tr>
<th>Cpd 1: Nitracaine: +ESI EIC (309.1809, 310.1848, 311.1897, 312.1932, 313.1967)</th>
</tr>
</thead>
</table>

MS Zoomed Spectrum

| Cpd 1: Nitracaine: +ESI Scan (rt: 5.842-6.663 min, 65 scans) Frag=125.0V Nitracaine_1443-16_T..
|---|

MS Spectrum Peak List

<table>
<thead>
<tr>
<th>Obs. m/z</th>
<th>Charge</th>
<th>Abund</th>
<th>Formula</th>
<th>Ion/Isotope</th>
</tr>
</thead>
<tbody>
<tr>
<td>309.1809</td>
<td>1</td>
<td>748231.5</td>
<td>C16 H24 N2 O4</td>
<td>(M+H)+</td>
</tr>
<tr>
<td>310.1843</td>
<td>1</td>
<td>1568059.69</td>
<td>C16 H24 N2 O4</td>
<td>(M+H)+</td>
</tr>
<tr>
<td>311.1897</td>
<td>1</td>
<td>294586.44</td>
<td>C16 H24 N2 O4</td>
<td>(M+H)+</td>
</tr>
<tr>
<td>312.1932</td>
<td>1</td>
<td>37363.17</td>
<td>C16 H24 N2 O4</td>
<td>(M+H)+</td>
</tr>
<tr>
<td>313.1967</td>
<td>1</td>
<td>3619.12</td>
<td>C16 H24 N2 O4</td>
<td>(M+H)+</td>
</tr>
</tbody>
</table>

--- End Of Report ---
### Peak Integration Report

<table>
<thead>
<tr>
<th>No.</th>
<th>Time (min)</th>
<th>Peak Name</th>
<th>Peak Type</th>
<th>Area (µS·min)</th>
<th>Height (µS)</th>
<th>Amount (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,00</td>
<td>8,09</td>
<td>Chloride</td>
<td>BMB</td>
<td>16,42</td>
<td>79,06</td>
<td>n.a.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TOTAL:</td>
<td></td>
<td>16,42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79,06</td>
<td></td>
<td>0,00</td>
</tr>
</tbody>
</table>

**Sample Name:** Nitracaine_1443-16_IC  
**Injection Vol.:** 25,00  
**Injection Type:** Unknown  
**Program:** ANIONI  
**Operator:** kemija  
**Inj. Date / Time:** 24-jun-2016 / 16:02  
**Run Time:** 42,00

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**Graph Description:**
- Peak 1: Chloride at 8.09 min with an area of 16.42 µS·min and a height of 79.06 µS.
- Peak 2: At 16.90 min, with an area and height not specified.

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**Notes:**
- **ANIONI** program used.
- **Dilution Factor:** 1,0000.
- **Operator:** kemija.
- **Injection Date / Time:** 24-jun-2016 / 16:02.
- **Run Time:** 42,00 minutes.
REPORT

Sample ID: 1443-16

Our notebook code: P-1443-16

NMR sample preparation: 15 mg dissolved in 0.7 mL CDCl₃

NMR experiments: ¹H, ¹³C, ¹H–¹H gs-COSY, ¹H–¹³C gs-HSQC, ¹H–¹³C gs-HMBC, ¹H–¹⁵N gs-HMBC.

Proposed structure:

![Proposed Structure Image]

Chemical name: N,N-diethyl-2,2-dimethyl-3-((4-nitrobenzoyl)oxy)propan-1-aminium cation

Comments: - Structure elucidation based on 1D and 2D NMR spectra
- Sample is pure as evident by NMR.

Supporting information: Copies of ¹H and ¹³C NMR spectra

Author: Prof. Dr. Janez Košmrlj, Doc. Dr. Krištof Kranjc

Date of report: August 24, 2016

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Current Data Parameters
NAME          P-1443-16
EXPNO                 1
PROCNO                1

F2 - Acquisition Parameters
Date_          20160807
Time               4.10
INSTRUM           spect
PULPROG 5 mm PABBO BB-
PULPROG zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                2
SWH           10000.000 Hz
AQ            3.2768500 sec
RG                 80.6
DW               50.000 usec
DE                6.50 usec
TE                300.0 K
D1          1.00000000 sec
D11                   1

======== CHANNEL f1 ========
SFO1        500.1330885 MHz
NUC1                1H
P1                 8.90 usec
PLW1        26.00000000 W

F2 - Processing parameters
SI                65536
SF          500.1299960 MHz
WDW                  EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.00

--- CHANNEL f1 ---------
SF01          500.1330885 MHz
NUC1                1H
P1                 8.90 usec
PLW1        26.00000000 W
PLW12        0.32179001 W
PLW13        0.16186000 W

--- CHANNEL f2 ---------
SF02          125.7703637 MHz
NUC2                13C
CPDPRG[2        waltz16
PCPD2             80.00 usec
PLM2        26.00000000 W
PLM12        0.32179001 W
PLM13        0.16186000 W

F2 - Processing parameters
SI                32768
SF          125.7577860 MHz
WDW                  EM
SSB      0
LB                 0.30 Hz
GB       0
PC                 1.40